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Structure, Biosynthesis, and Physicochemical Properties of Archaebacterial Lipids

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INTRODUCTION

A series of recent phylogenetic studies, based on 16S ribosomal ribonucleic acid (RNA) composition (2, 33, 84, 104, 105) and other general biochemical features (33, 105) such as RNA polymerase (71, 81, 86, 113, 116), translation system (10, 54, 55, 80, 85), transfer RNA (44, 56, 58, 59), 5S ribosomal RNA (32, 69, 70), cell wall (21, 50–52, 77, 84, 103), and lipids (25, 26, 28, 53, 60, 67, 72, 98), reveals the existence of a new group of microorganisms named archaebacteria. Some features, e.g., their small size and structural simplicity, the absence of a nuclear membrane and organelles, and low deoxyribonucleic acid content, indicate that archaebacteria are procaryotic (33, 84, 105). In contrast, it has been proposed that archaebacteria are closely related to the ancestor eucaryotes (79, 100) since several characteristics, such as the presence of histone-like proteins (42, 87, 96), the nature of their translation system (54, 55, 85), and the sequence of their 5S RNA (46) and that of ribosomal proteins (73), are considered to be typical of eucaryotes.

This body of results leads us to consider that archaebacteria are as far from eubacteria as they are from eucaryotes (103). Archaebacteria are quite interesting microorganisms from the point of view of the early evolution of life; indeed, their name was given to underline the hypothesis that these organisms were the dominant inhabitants of the earth in early ancient times (2, 33, 104, 105). In this respect, archaebacteria, as a distinct primary kingdom, are very important as they give us some indications of the early events in the evolution of cells, thus contributing to a better understanding of the universal ancestor.

Archaebacteria are characterized by a wide metabolic diversity and a high degree of morphological variability that is roughly comparable to that found in eubacteria. In fact, this group includes aerobes, anaerobes, autotrophs, heterotrophs, thermophiles, acidophiles, phototrophs, cocci, rods, and disk-shaped and pleiomorphic forms (1, 6, 7, 9, 16, 22, 49, 74, 77, 83, 93, 104, 105, 111, 112, 114, 115). Archaebacteria are classified into three major phenotypes; halophiles, methanogens, and thermophiles (105). They thrive in environments that would normally kill many other known organisms; in fact, they are now segregated into a few peculiar ecological niches, such as saturated brine for extreme halophiles (35, 49, 97), stagnant water, rumen of animals, and hydrothermal environments for methanogens (107, 108), and thermal habitats for extreme thermophiles (104).

The halophiles comprise five genera: *Halobacterium*, *Halococcus*, *Haloarcula*, *Natronobacterium*, and *Natronococcus*, with 17 different species. Their growth pH is near neutrality for *Halobacterium*, *Halococcus*, and *Haloarcula* and strongly alkaline for *Natronococcus* and *Natronobacterium*; all are mesophiles (35, 36, 49, 82, 83, 97). The methanogenetic phenotype encompasses 11 genera: *Methanolobus*, *Methanoplanus*, *Methanogenium*, *Methanospirillum*, *Methanomicrobium*, *Methanotrix*, *Methanosarcina*, *Methanococcus*, *Methanobacterium*, and *Methanothermus*, with 26 species, including 5 that are thermophilic (1, 13, 48, 57, 91, 94, 102, 109, 110). Among the thermophiles, 13 different microorganisms have so far been classified, belonging to eight different genera: *Thermococcus*, *Thermoproteus*, *Desulfurococcus*, *Thermofilum*, *Sulfolobus*, *Pyrodictium*, *Thermodiscus*, and *Thermoplasma*; they are anaerobic or aerobic, and all live at pH values below 7, at an optimal temperature, ranging from 60 to 105°C (4, 6–9, 16, 22, 34, 88, 92, 93, 99, 111–115, 117).

LIPID STRUCTURE OF ARCHAEBACTERIA

All membrane lipids of the archaebacteria so far identified are characterized by unusual structural features, which can be considered to be specific taxonomic markers of this group of microorganisms. In fact, while all living organisms so far known have membranes based on ester linkages, formed by the condensation of alcohols and fatty acids, archaebacteria have lipids based on ether linkages. These molecules are formed by condensation of glycerol or more complex polyols with isoprenoid alcohols containing 20, 25, or 40 carbon atoms. Moreover, it is worth noting that all glycerol ethers in archaebacteria contain a 2,3-di-*O*-*sn*-glycerol, which is un-

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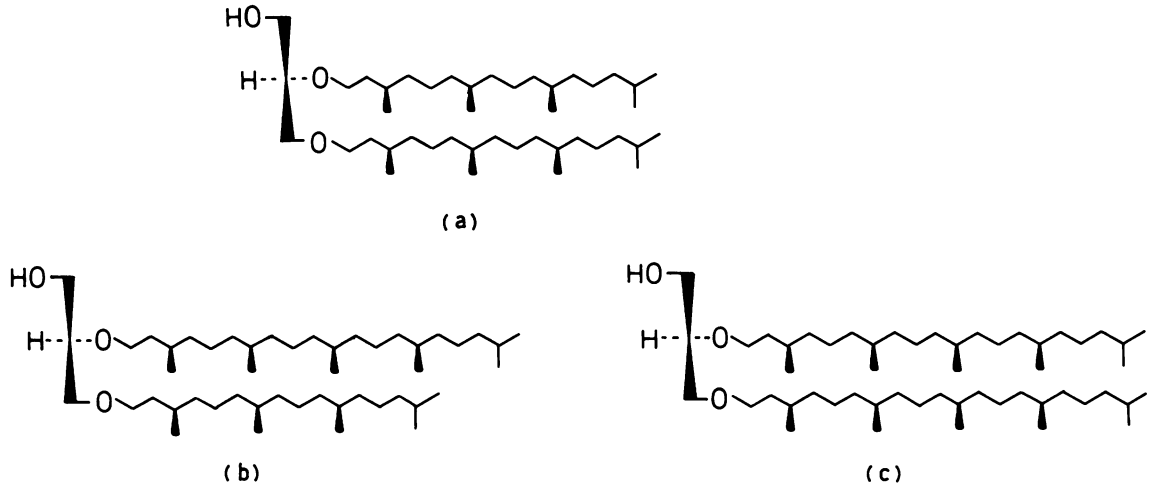


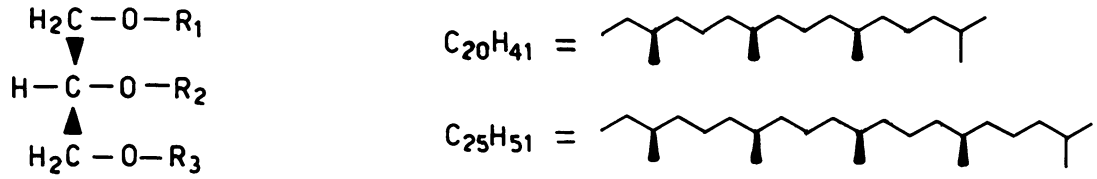
FIG. 1. 2,3-Di-*O*-phytanyl-*sn*-glycerol, basic component of membrane lipids of all halophilic archaebacteria (a); 2-*O*-sesterterpanyl-3-*O*-phytanyl-*sn*-glycerol (b) and 2,3-di-*O*-sesterterpanyl-*sn*-glycerol (c), basic components of membrane lipids of some halophilic archaebacteria.

usual, since the glycerol in the naturally occurring glycerophosphatides or diacylglycerols is known to have an *sn*-1,2 stereochemistry (29, 53, 61).

Halophiles

The basic structural elements of all complex lipids present in the halophilic archaebacteria are presented in Fig. 1. All extremely halophilic archaebacteria possess lipids based on 2,3-di-*O*-phytanyl-*sn*-glycerol (Fig. 1a). In extremely alkaliphilic red halophiles living at pH 10 (97) and in some strains of neutral halophiles (83), the lipids are based also on the structural types shown in Fig. 1b and c and contain the C_{25} sesterterpanyl chain (27, 28, 83). The complex lipids of halophiles have been extensively studied (53, 83); they derive from the structures shown in Fig. 1, the free —OH group of which is linked to different polar groups, giving rise to a large range of molecules, as shown in Fig. 2.

The compounds shown in Fig. 2a and b are the major



Compound	R_1	R_2	R_3
a	$C_{20}H_{41}$ $C_{20}H_{41}$ $C_{25}H_{51}$	$C_{20}H_{41}$ $C_{25}H_{51}$ $C_{25}H_{51}$	sn-3-(1-phospho)-glycerol-P
b	$C_{20}H_{41}$ $C_{20}H_{41}$ $C_{25}H_{51}$	$C_{20}H_{41}$ $C_{25}H_{51}$ $C_{25}H_{51}$	sn-3-glycerol-P
c	$C_{20}H_{41}$	$C_{20}H_{41}$	sn-3-(1-sulfo)-glycerol-P
d	$C_{20}H_{41}$	$C_{20}H_{41}$	β-gal-3-sulfate-(1→6)-α-man-(1-2)-α-glc
e	$C_{20}H_{41}$	$C_{20}H_{41}$	β-gal-(1→6)-α-man-(1→2)-α-glc
f	$C_{20}H_{41}$	$C_{20}H_{41}$	β-glc-(1→6)-α-man-(1→2)-α-glc

FIG. 2. Structures of polar lipids from halophilic archaebacteria.

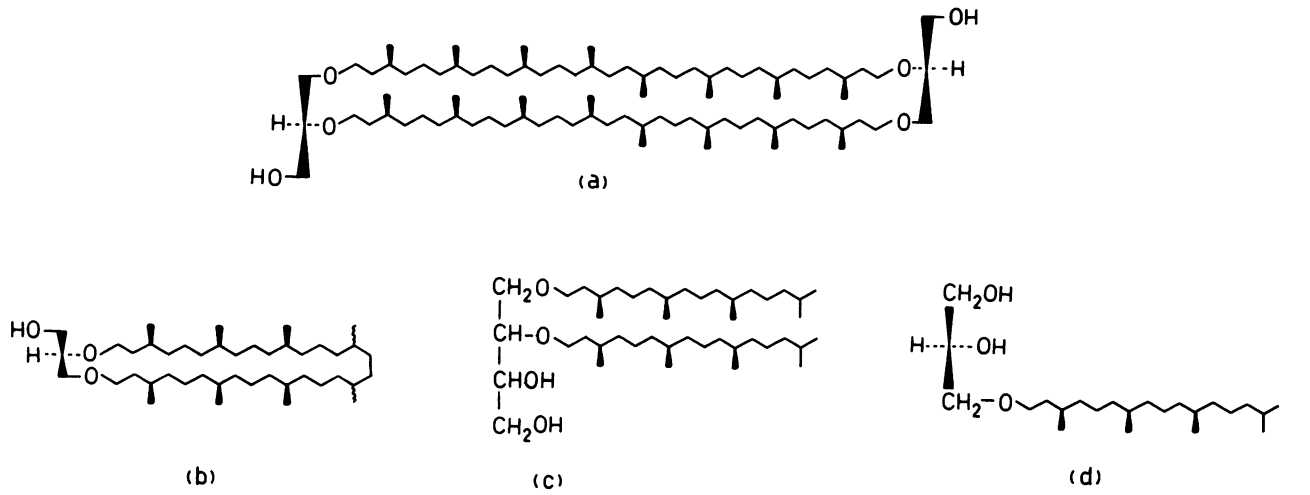


FIG. 3. Isoprenoid ethers, backbone of complex lipids of methanogenic archaebacteria. (a) Di-biphytanyl-diglycerol-tetraether; (b) macrocyclic diether; (c) tetritol-diphytanyl-diether; (d) 3-*O*-phytanyl-*sn*-glycerol.

phospholipids occurring in halophilic archaebacteria (53). While the isoprenoid moieties of these lipids in neutrophilic halophiles are phytanyl chains, the isoprenoid residues in alkaliphilic halophile phospholipids are based also on sesterterpanylic component(s) (83, 97). It is worth noting that the configuration of both glycerol residues in the structures in Fig. 2a to c is opposite that found in the corresponding classic ester lipids (53). Complex lipids (Fig. 2c to f) occur in neutrophilic halophilic archaebacteria only (53, 83, 97).

Methanogens

In most cases, the membrane lipids of methanogens are based on the diphytanyl-glycerol-diether (Fig. 1a) and the di-biphytanyl-diglycerol-tetraether (Fig. 3a), formed by dimerization of two diphytanyl-glycerol-diethers where head-to-head linkage between the terminal methyls occurs (60, 61, 67, 72, 98).

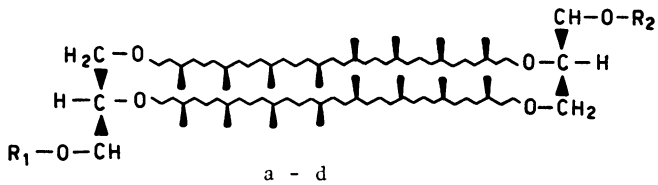
In the *Methanococcus* genus only, 2,3-di-*O*-phytanyl-*sn*-glycerol (Fig. 1a) occurs as the backbone of complex lipids, but in *Methanolobus* species, in addition to this diether, 2-*O*-sesterterpanyl-3-*O*-phytanyl-*sn*-glycerol (Fig. 1b) also is present (W. D. Grant, G. Pinch, J. E. Harris, M. De Rosa, and A. Gambacorta, J. Gen. Microbiol., in press).

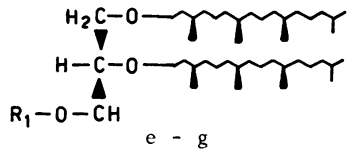
Moreover, lipids of *Methanococcus jannaschii* are based mainly on the macrocyclic diether of Fig. 3b (13), formed by the head-to-head linkage between the terminal methyl groups of the two phytanyl residues present in the 2,3-di-*O*-phytanyl-*sn*-glycerol (Fig. 1a). Finally, in Fig. 3c and d two new structural types, present in *Methanosarcina barkeri*, are shown. The first is the tetritol-diphytanyl-diether (Fig. 3c); the second is 3-*O*-phytanyl-*sn*-glycerol (Fig. 3d). In *Methanosarcina* spp. di-biphytanyl-diglycerol-tetraethers with cyclopentane rings in the isoprenoid C_{40} chains also are present (see Fig. 5c, d, and h) (M. De Rosa, A. Gambacorta, V. Lanzotti, A. Trincone, J. E. Harris, and W. D. Grant, Biochim. Biophys. Acta, in press).

Structures of complex lipids from methanogens, extensively studied in *Methanospirillum hungatei* (60, 61), are presented in Fig. 4. It is worth noting that the phosphoryl-1-*sn*-glycerol residue, occurring in lipids shown in Fig. 4g, is diastereoisomeric with the counterpart in the phospholipids of extreme halophiles shown in Fig. 2a to c.

Thermophiles

The situation in thermophilic archaebacteria is more complex; in these microorganisms, there are many types of basic structural unities (Fig. 5) which give rise to various complex lipids (26). The available data on the lipid composition of the





Compound	R_1	R_2
a	α-glcp-(1→2)-β-galf	sn-3-glycerol-P
b	"	"
e	"	/
c	β-galf-(1→6)-β-galf	sn-3-glycerol-P
d	"	"
f	"	/
g	sn-1-glycerol-P	

FIG. 4. Structure of polar lipids from *Methanospirillum hungatei*.

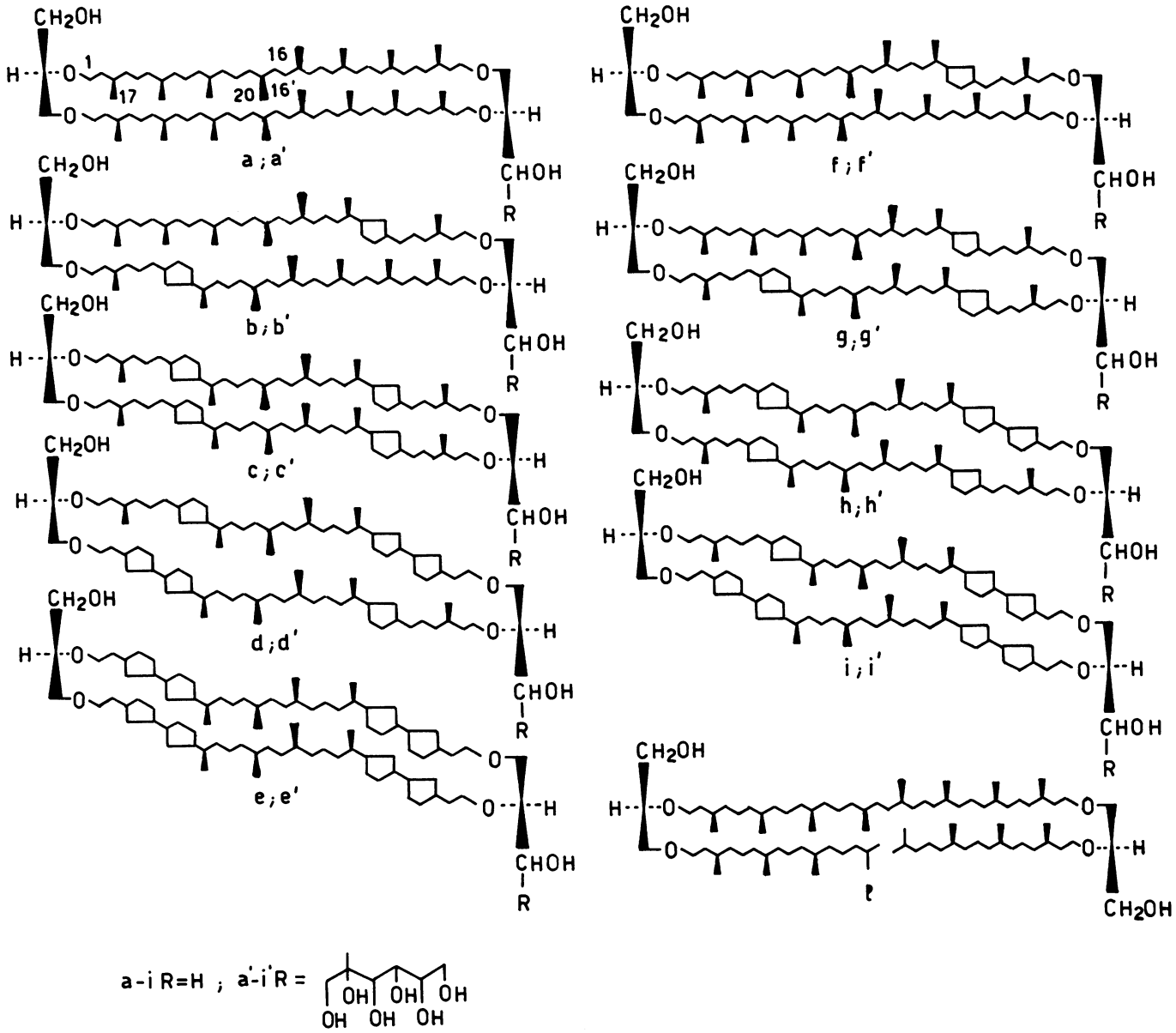


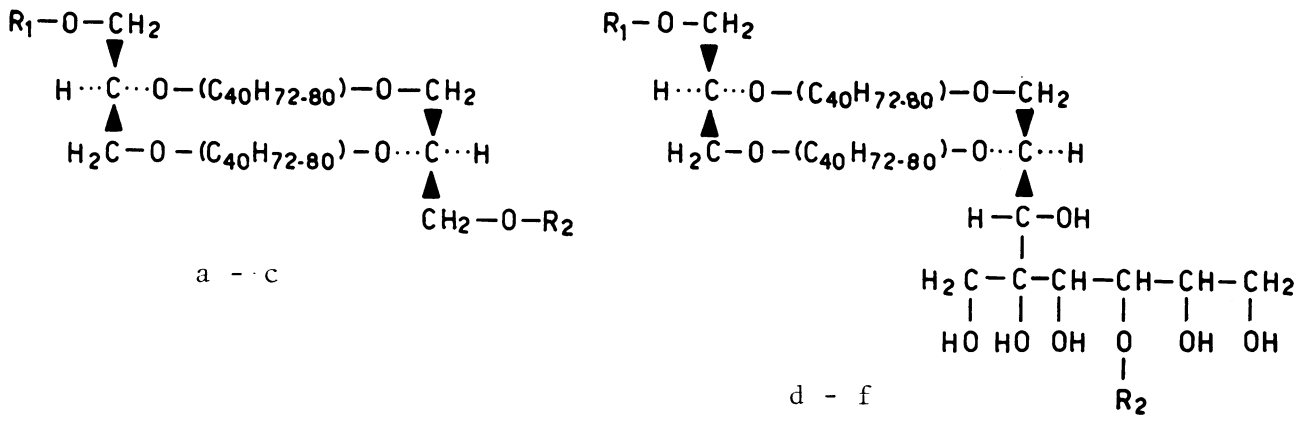
FIG. 5. Structural types of isoprenoid ethers, backbone of complex lipids of thermophilic archaebacteria. See text for details.

genera *Desulfurococcus*, *Thermoproteus*, *Thermofilum*, and *Pyrodictium* indicate the occurrence of ether lipids based on C_{20} phytanyl chains and C_{40} biphytanyl chains (93, 111, 114, 115).

More extensive studies have been performed on lipids of the *Sulfolobus* species, which are based essentially on tetraethers (Fig. 5). These tetraethers can be divided into two classes: the first, R = H, may simply be called glycerol-dialkyl-glycerol-tetraethers (GDGT; Fig. 5a to i) and is formed by two *sn*-2,3-glycerol moieties bridged through ether linkages by two isoprenoid C_{40} diols, formally derived from ω,ω' linkages of two *O*-phytanyl residues. The structural organization of the second class of molecules in which R = $C_6H_{13}O_6$ (Fig. 5a' to i') is similar, even though a more complex branched nonitol replaces one of the glycerols; these molecules have been named glycerol-dialkyl-nonitol tetraethers (GDNT). Finally, as minor components, 2,3-di-*O*-phytanyl-*sn*-glycerol (Fig. 1a) and glycerol-trialkyl-glycerol tetraether (Fig. 5e) (26) are present; this last is based on two phytanyl chains and one 16-16' C_{40} isoprenoid.

The C_{40} components in these lipids differ in the additional feature of containing up to four cyclopentane rings; the degree of cyclization in the biphytanyl components is sensitive to environmental parameters, such as temperature. In fact, it has been shown that the extent of cyclization in the C_{40} components increases when *Sulfolobus solfataricus* is grown at increasing temperatures (20). At optimal growth temperature (87°C) the major species present are tetraethers (c,c'; d,d'; and h,h' in Fig. 5) (26).

Thermoplasma sp. lipids are based essentially on the GDGT (Fig. 5a, b, and c) and on 2,3-di-*O*-phytanyl-*sn*-glycerol, as a minor component (64, 66, 67), while the GDNT species are completely absent. Until now the only thermophilic archaebacterium without GDGT and GDNT, as the backbone of complex lipids, was *Thermococcus celer*, which instead contains complex lipids, based on 2,3-di-*O*-



Compound	R_1	R_2
a	H	β-glcp-β-galp
b	inositol-P	H
c	inositol-P	β-glcp-β-galp
d	H	β-glcp
e	H	β-glcp-sulfate
f	inositol-P	β-glcp

FIG. 6. Complex lipids of *S. solfataricus*; structural details on C_{40} chains are given in Fig. 5.

phytanyl-*sn*-glycerol (M. De Rosa, A. Gambacorta, A. Trincone, A. Basso, W. Zillig, and I. Holz, personal communication).

Structures of the complex lipids of the thermophilic archaebacteria, extensively studied in the genus *Sulfolobus*, are shown in Fig. 6 (24, 25, 62, 65, 67).

ARCHAEBACTERIAL ORIGIN OF FOSSILS IN ANCIENT SEDIMENTS

Mounting evidence indicates that a large fraction of isoprenoid hydrocarbons in sedimentary rocks as old as Precambrian Era are of archaebacterial origin (45). Albrecht and colleagues (12, 75) isolated phytane head-to-head-linked biphytanyl and head-to-head-linked C_{40} isoprenoid containing one 1,4-cyclopentane ring in the kerogen of the 50-million-year-old lacustrine Eocene Messel oil shale. Moreover, Brassell et al. (5) have found a 2,6,10,15,19-pentamethyl-eicosane, a known component of methanogens, in various marine sediments of the Recent to Cretaceous age. More recently, ether lipids typical of archaebacteria, such as the tetraether shown in Fig. 3a, have been found in polar fractions of several recent and ancient sediments and petroleum of various origins and ages (11). The results provide evidence of the widespread occurrence of archaebacterial lipids in geological organic matter and show that the polar lipids of organisms are at least partially preserved in the sediments, even through an advanced degree of maturation, beyond the stage of petroleum generation. This reflects the stability of these lipids, in particular, of their ether linkages, under geological conditions.

BIOSYNTHETIC STUDIES OF ARCHAEBACTERIAL LIPIDS

The C_{20} and C_{40} components of the complex lipids of archaebacteria are efficiently and selectively labeled following the uptake of either labeled acetate or mevalonate (18, 53, 65, 66).

Extensive studies of the labeling patterns from [1-^{13}C]- and, particularly, [1,2-$^{13}C_2$]acetate in biphytanyl components of *Sulfolobus* spp. lipids have been quite informative because they establish the overall applicability of the classic route from acetate to isoprene units in which C-2 of the intermediate mevalonate gives rise to the in-chain CH_2 moiety of the *trans*-isoprene unit (18, 23).

Isoprenoid biosynthesis in thermophilic and methanogenic archaebacteria includes the formation of C-C bonds either within or between prenyl chains; both processes are peculiar to the archaebacteria. They are as follows: (i) head-to-head coupling of two geranyl-geranyl residues with reduction to form biphytanyls in methanogens and thermophiles; (ii) cyclization within coupled geranyl-geranyl residues with reduction to form five-membered cyclic biphytanyls in thermophiles.

The process of head-to-head C_{20}-C_{20} coupling is particularly striking and has no parallel in other fields of terpene biochemistry. However, there is no direct evidence as to its mechanism. Experiments on incorporation of [$^{13}C_2$]acetate in *Sulfolobus* spp. establish (23) that the coupling is between the two carbons derived from C-2 of mevalonate. At present, there is no direct information as to whether the head-to-head coupling is between C_{20} chains ether linked to glycerol or

between the C_{20} precursors themselves. However, indirect evidence favors the former.

The structural regularities of tetraethers (Fig. 5) (30) are in accord with the supposition that cyclizations occur in the axially symmetric tetraethers rather than in the free C_{20} or C_{40} components. In contrast, it is difficult to see how ether formation from the free, partially cyclized C_{40} components could take place with the requisite specificity.

Formation of ether linkage was first investigated by Kates and co-workers (53) in the formation of diphytanyl glycerol by *Halobacterium* spp. They showed that the glycerol moiety has the *sn*-2,3 configuration, which is opposite to that of the diacyl glycerol in conventional ester lipids (*sn*-1,2). Incorporation experiments with variously labeled [^{14}C]- and [^{3}H]glycerol as the precursor showed that, (i) as expected from normal glycolytic pathways, both [1(3)-^{14}C]- and [1(3)-^{3}H]glycerol gave substantial activity in the phytanyl chains by way of acetyl-coenzyme A, and also in the sugars of complex lipids by way of triose, but [2-^{3}H]glycerol labeled neither the phytanyl chains nor the lipid sugars, and (ii) in the glycerol moiety of the ether lipids, label from [2(3)-^{14}C, 1(3)-^{3}H]glycerol was incorporated intact, that is, with 100% conservation of the initial ^{3}H/^{14}C ratio. In contrast, label from [1(3)-^{14}C, 2-^{3}H]glycerol was incorporated with virtually complete (90%) loss of tritium. Although the authors regarded the loss of tritium from C-2 of glycerol diether formation as a significant feature of the etherification step, more recent experiments with *Sulfolobus* spp. cast doubt on this and favor the alternative explanation that the loss of tritium is due to the interconversion of glycerol (or glycerol phosphate or both) and dihydroxyacetone (or phosphate or both).

In experiments on tetraether formation by *Sulfolobus* spp., both [U-^{14}C, 1(3)-^{3}H]- and [U-^{14}C, 2-^{3}H]glycerol were selectively incorporated in the glycerol moieties of tetraethers with high efficiency and without change in the ^{3}H/^{14}C ratio (29).

Thus, in *Sulfolobus* spp. the ether-forming step can occur without any loss of hydrogen from any of the glycerol carbons and without the intervening formation of any oxidized derivative of the glycerol. Given the abundantly demonstrated ability of geranylgeranyl pyrophosphate and similar allyl pyrophosphates to act as alkylating agents in other biosynthetic mechanisms, direct ether formation from glycerol (or, facilitated by neighboring group deprotonation, from glycerol phosphate) presents no conceptual difficulties. On the other hand, such alkylating reactivity would be lessened in a nonallyl (phytanyl) pyrophosphate, and we regard this as further evidence for the conclusion that ether formation precedes reduction in the isoprenoid part of ether lipids in *Sulfolobus* spp. According to this hypothesis, the unusual configuration of the chiral center in the glycerol moiety would depend, in this microorganism, on the stereospecific nature of the alkylation step. The metabolic fate of both labeled glycerols in the isoprenoid moiety of *Sulfolobus* spp. lipids is similar to that reported for *Halobacterium* spp. by Kates and Kushwaha (53).

Further biosynthetic studies on *Sulfolobus* spp. have been performed to determine the origin of the branched nonitol characteristic of GDNT lipids (Fig. 5a′ to i′) (19).

Plausibly, this polyol could be formed by a variety of aldol- or acetoin-type condensations between a triose and a hexose precursor, followed by reduction, as shown in Fig. 7 (without implications as to stereochemistry, phosphorylation, etc.).

The specific incorporation of labeled glucose and its metabolic equivalent fructose in C-4 to C-9 of the nonitol skeleton (Fig. 7) is in accordance with this hypothesis. In particular, the observation that [U-^{14}C, 1(3)-^{3}H]glycerol labels the nonitol moiety, with 70% ^{3}H retention, while [U-^{14}C, 2-^{3}H]glycerol was incorporated with a complete loss of ^{3}H and evidence of a selective localization of the radioactivity with both precursors, at the level of C-1 to C-3 of nonitol, favor a biosynthetic route that implies, first, the oxidation of a secondary carbon of glycerol and then, the removal of one hydrogen from this oxidized intermediate in the assembly of the C-9 nonitol skeleton (De Rosa et al., unpublished results).

PHYSICOCHEMICAL STUDIES OF BIPOLAR LIPIDS

When *S. solfataricus* is grown at various temperatures, the lipids show a degree of cyclization of biphytanyl components which increases with increasing environmental temperature (20). Differential scanning calorimetry indicates, indeed, the presence of a variety of transitions, the critical temperatures of which depend on the number of cyclopentane rings (40; A. Gliozzi, G. Paoli, D. Pisani, F. Gliozzi, M. De Rosa, and A. Gambacorta, submitted for publication). Accordingly, differential scanning calorimetry measurements performed on the native lipid mixture of *Thermoplasma acidophilum*, characterized by a much lower degree of cyclization (64), show a remarkably lower temperature of the gel-liquid crystal transition (3). The nature of the various transitions has been investigated also by X-ray diffraction analysis (43). The main conclusions that stem from this study are that, as with other lipids, a remarkable number and variety of phases are observed over a temperature-concentration range close to physiological conditions. The possibility is discussed that this polymorphism reflects a fundamental property of lipids, closely related to their physiological role. Information on the dynamics of the lipid molecules is also provided by electron paramagnetic resonance spin label studies either in vivo, in *Thermoplasma* sp. membranes (101, 106), or in lipid extracts from *S. solfataricus* (9a). In the latter case, a marked difference has been observed between correlation times of the symmetric GDGT and asymmetric GDNT lipids, the latter being larger by a factor of up to 10^3. This is due to the presence of the nonitol polar heads, which can form a large number of hydrogen bonds (37). It is worth noting that GDNT lipids do not form closed vesicles (68); only addition of at least 25% monopolar lipids (in particular, phosphatidylcholine) leads to the formation of small unilamellar liposomes.

The symmetrical GDGT lipid does not form stable black membranes, presumably because of the low polarity of the two OH groups at the ends of the tetraether. By contrast, the asymmetrical GDNT lipid does form planar black membranes (39), the properties of which are discussed below. Structural changes have also been detected in polar lipids of *Halobacterium halobium* by nuclear magnetic resonance studies (17). The latter work also indicates that, due to the presence of the methyl side groups in the alkyl chains, the segmental motion at the tertiary carbons is hindered.

ORGANIZATION OF BIPOLAR LIPIDS IN THE PLASMA MEMBRANE

Many models have been proposed to describe the architecture of the plasma membrane. Among them, the most important historically is the Danielli-Davson model or the so-called unit membrane model (15). A deeper knowledge of the structure and molecular dynamics of the membrane

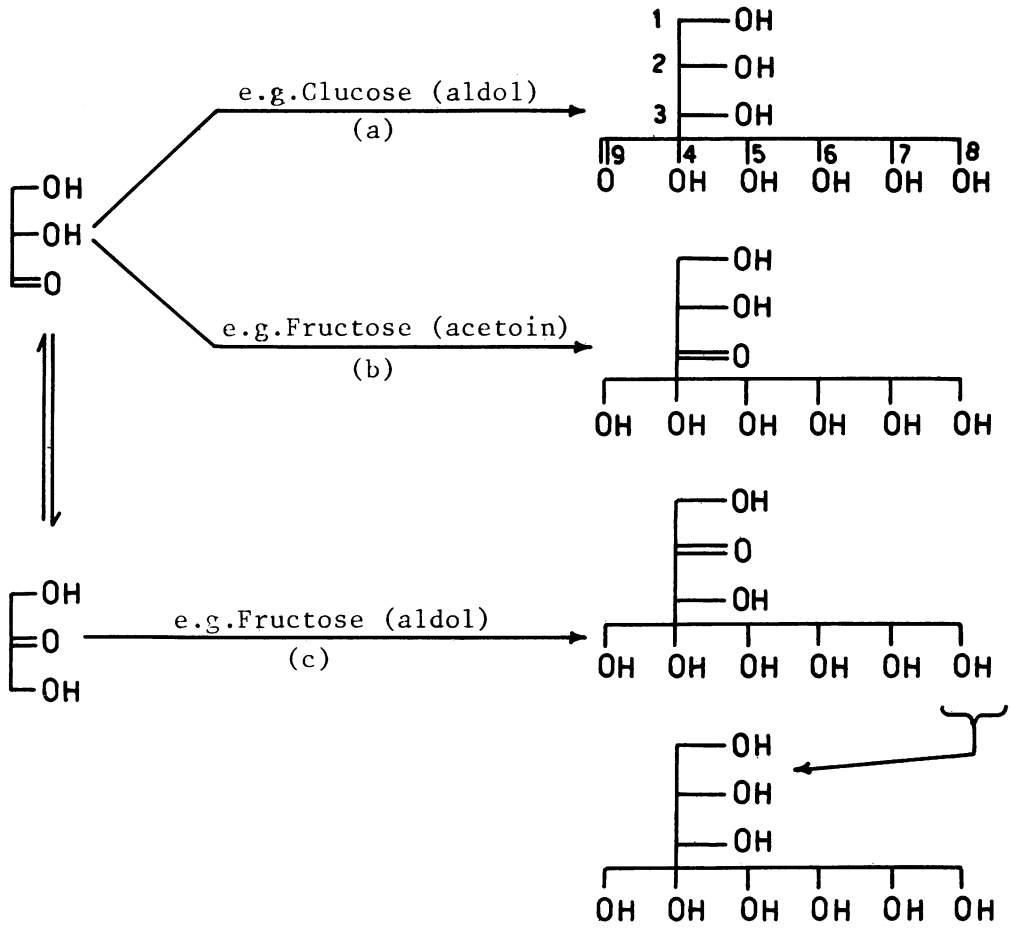


FIG. 7. Hypothesized mechanism of nonitol biosynthesis.

components has led to the popular fluid mosaic model, proposed in 1972 by Singer and Nicholson (89). More recently, Dolowy (31), on the basis of biochemical data, has suggested that all lipids of erythrocytes are covered, more or less accurately, by protein molecules. In spite of the great differences among these models, all of them assume as basic structure of the plasma membrane a bilayer lipid leaflet. This assumption has been supported by various physical techniques, such as X-ray diffraction, nuclear magnetic resonance, and Raman spectroscopy. Only a few processes, including cell fusion, exo- and endocytosis, protein insertion, and orientation, are difficult to reconcile with an inviolate bilayer structure (14). Thus, besides large bilayer regions, other forms of organization, related to the polymorphic behavior of the lipids, might exist. When the lipid molecules have two different polar head groups at opposite ends, as in the case of thermophilic archaebacteria belonging to the *Sulfolobus* genus, a monomolecular layer in which each molecule spans the entire thickness of the membrane might occur. Such an arrangement would be the first example of a membrane the lipid structure of which lacks a midplane region. Indirect evidence, such as the structure and dimensions of lipids themselves, the absence of a preferential fracture plane upon freeze-fracturing, and the extreme rigidity of the thermophilic archaebacteria membranes, supports the concept of a monolayer organization of the lipids (41, 63). Two direct experiments performed on intact cell membranes and on black lipid films represent further evidence for this hypothesis. The first one is the determination of glycosidic linkages exposed to the external surface of the cell membrane (24). Towards this end, intact cells of *S. solfataricus* were treated with a nonpenetrating reactant (a mixture of glycosidases from a marine gastropod) able to permeate only the cell wall and to hydrolize the glycosidic bonds of the membrane lipids of the microorganism. Notice that in membranes of *Sulfolobus* species the lipids with glycosidic linkages on one of the polar heads constitute 92% of the total complex lipids.

Analysis of purified lipids extracted from the cells indicates that, after 120 h, at least 82% of the total lipids are hydrolized. These results indicate a monolayer organization of the lipids, with glycosidic linkages exposed outside.

The second set of experiments has been performed on black lipid films of GDNT (37–41). They include zero-voltage capacitance (measured by dispersing the lipids in various solvents), voltage-dependent capacitance, current-voltage relationships, and relaxation measurements (A. Gliozzi, S. Bruno, T. K. Basak, M. De Rosa, and A. Gambacorta, System. Appl. Microbiol., in press). These experiments have been carried out over a wide range of temperatures (10 to 80°C). This body of results is also consistent with a monolayer organization of the lipids.

These findings are very important from an evolutionary point of view in that this new model of molecular membrane architecture is the first example of an alternative topologic solution in a structure that, until now, has appeared to

remain basically unchanged, from the simplest procaryotes up to humans.

In the plasma membrane this organization may accomplish a twofold task. First, it confers stability on the membrane; second, it may constitute a barrier against the diffusion of hydrogen ions into the cell. Indeed, the cell must withstand a pH gradient of 4 to 5 pH units. The nonitol polar heads, linked with sugars and endowed with a large dipole moment, are located towards the outside of the cell; consequently, a higher dipole potential barrier at the external surface with respect to the cytoplasmic side is developed (40; Gliozzi et al., in press). A further contribution to the passive proton exclusion is given by the positive value of the membrane potential, whereas most other cells are negative inside. In *S. acidocaldarius* the membrane potential measured with labeled ions of triphenylmethylammonium and thiocyanate gives 30 to 60 mV (76). A much higher value (109 to 125 mV positive inside) is found in *Thermoplasma acidophilum* (47).

Little is known about archaebacterial membrane proteins, with the exception of bacteriorhodopsin, which constitutes 90% of the purple membrane of *H. halobium* (79, 95). Some further information on membrane proteins derives from *Thermoplasma acidophilum*, because of the greater simplicity of its plasma membrane, devoid of the cell envelope. Purified membranes can be obtained by sonication of cell suspensions at moderate ionic strength (0.05 M) and pH 5 (7, 63, 90). Their chemical composition by weight is 60% proteins, 25% lipids, and 10% carbohydrates. Gel electrophoresis has revealed that these membrane proteins are a heterogeneous mixture that possess an amino acid composition similar to that of mycoplasma membrane proteins (90). Thus, the percentage of proteins in archaebacterial membranes, in the very few cases known so far, is very high.

How are proteins inserted in a monolayer of bipolar lipids? A tentative picture has been given on the basis of electron paramagnetic resonance studies which have shown that, at physiological temperatures, the fluidity of the center portion of the hydrocarbon core is similar to that of an egg lecithyn bilayer, thus providing the proper microviscosity for protein insertion (9a). Furthermore, X-ray diffraction studies (43) have shown that unsubstituted glycerol, whose concentration is high in *S. solfataricus* complex lipids, can interact with hydrophobic molecules, thus providing new possibilities for interactions with proteins. It is also significant that the cell envelopes of most archaebacteria are made up of continuous layers of proteins, and it seems very restrictive to regard them as merely passive barriers.

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